REMARKS

The specification is amended solely for compliance with the sequence listing rules. Applicants hereby state that the amendment does not include new matter. Applicants hereby further state that the information recorded in computer readable form is identical to the written sequence listing.

It is believed that no additional fees or charges are required at this time in connection with the present application; however, if any fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Page 10, line 1:

The full-length cDNA of Fsβ-glucanase in a DNA template, such as the pJI10 plasmid as used in the preferred embodiment described herein, is amplified and introduced with a *Nco I* and an *EcoR I* restriction enzyme recognition sites at 5' and 3' ends, respectively, by using a PCR-based method. The two primers designed for introducing the *Nco I* and *Eco RI* sites are 5'TCACCACCATGGTTAGCGCAAAG-3' (SEQ ID NO: 7), and 5'GCCACGAATTCTGTTCAAAGTTC AC-3' (SEQ ID NO: 8), respectively. The PCR reaction is performed with a thermo-cycling program as follows: (94 °C, 5 min; 55 °C, 1 min, 72 °C, 1 min for 1 cycle), (94 °C, 1 min; 55 °C, 1 min, 72 °C, 1 min for 30 cycles), (94 °C, 1.5 min; 55 °C, 1.5 min, 72 °C, 10 min for 1 cycle). The resulting amplified DNA fragments are digested with *Nco I* and *Eco RI*, purified, and ligated onto the pET26b (+) vector which is pre-digested with *Nco I* and *Eco RI*. The sequence of Fsβ-glucanase can be confirmed by any conventional DNA sequencing methods, such as the chain termination method (Sanger, 1977). In this DNA construct, a *pel* B leading peptide at the N-terminus and extra 19 amino acid residues including 6X-histidine tag at the C-terminus to facilitate protein purification are included. The recombinant plasmid encoding for the wild-type enzyme is then transformed into *E. coli* BL21 (DE3) host.

Page 11, line 8-9:

The gene for 1,3-1,4-β-D-glucanase (PCR-TF-glucanase) can be truncated by using a PCR method, which uses Oligo A and Oligo B as a pair of specific primers and the full

length cDNA of Fsβ-glucanase in pJI10 as template. Oligo A: 5'-CAGCCGGCGATGGCCATGGTTAGCGCA-3' (SEQ ID NO: 9) and oligo B: 5'-CTGCTAGAAGAATTCGGAGCAGGTTCGTC-3' (SEQ ID NO: 10), are designed to amplify both strands of the gene corresponding to the amino acid sequence from methionine 1 to proline 248. The amplified DNA fragments are digested with *Nco I* and *Eco RI* and then ligated with a pET26b (+) vector (purchased from Novagen, WI, USA) which is pre-digested with *Nco I* and *Eco RI*, forming a recombinant plasmid containing a truncated Fsβ-glucanase gene. The truncated gene of Fsβ-glucanase in the recombinant plasmid can be confirmed by a chain termination DNA sequencing method (Sanger, 1977). In this DNA construct, a *pel* B leading peptide at the N-terminus and an extra 19 amino acid residues with a 6X-histidine tag at the C-terminus with respect to that of TF-glucanase sequence are included. Finally, the plasmid containing the truncated glucanase gene can then be transformed into *E. coli* BL21(DE3) host, purchased from Novagen, WI, USA. Of course, other gene truncation methods or agents may be used satisfactorily.

IN THE CLAIMS:

Please amend claims 11 and 15 as follows:

11. (Amended) A method of producing said truncated glucanase of claim 1, comprising:

(a) growing in a culture medium a bacterial strain [carrying a plasmid] containing a gene encoding for a wild-type 1,3-1,4-β-D-glucanase from *Fibrobacter succinogenes*,

[(b) adding to said culture medium an inducer to induce expression of said gene and continuing said growing of step (a),]

(b) [(c)] centrifuging said culture medium to produce a supernatant,

(c) [(d)] incubating said supernatant to produce said truncated glucanase, and

(d) [(e)] collecting and purifying said truncated glucanase from said supernatant.

15. (Amended) A method of producing said truncated glucanase of claim 1, comprising:

(a) amplifying a DNA fragment using a PCR method from a DNA template containing a gene encoding for a wild-type glucanase from *Fibrobacter succinogenes*, said DNA fragment substantially corresponding to a portion of said gene,

(b) subcloning said amplified DNA fragment in an expression vector,

(c) transferring said expression vector harbouring said DNA fragment into a [bacterial] host strain,

(d) growing said [bacterial] host strain in a culture medium for a period of time and inducing expression of said DNA fragment, with or without adding an inducer, to produce a sufficient amount of protein products, and

(e) collecting and purifying protein expression products from said culture medium.